

University of Dundee

## Potency of extracts from selected Egyptian plants as inducers of the Nrf2-dependent chemopreventive enzyme NQO1

Hamed, Ahmed R; Hegazy, Mohamed-Elamir F; Higgins, Maureen; Mohamed, Tarik A; Abdel-Azim, Nahla S; Pare, Paul W

*Published in:*  
Journal of Natural Medicines

*DOI:*  
[10.1007/s11418-016-0994-0](https://doi.org/10.1007/s11418-016-0994-0)

*Publication date:*  
2016

*Document Version*  
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

### *Citation for published version (APA):*

Hamed, A. R., Hegazy, M-E. F., Higgins, M., Mohamed, T. A., Abdel-Azim, N. S., Pare, P. W., & Dinkova-Kostova, A. T. (2016). Potency of extracts from selected Egyptian plants as inducers of the Nrf2-dependent chemopreventive enzyme NQO1. *Journal of Natural Medicines*, 70(3), 683-688. <https://doi.org/10.1007/s11418-016-0994-0>

### General rights

Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

## Potency of extracts from selected Egyptian plants as inducers of the NRF2-dependent chemopreventive enzyme NQO1

Ahmed R. Hamed<sup>1,\*</sup> • Mohamed-Elamir F. Hegazy<sup>1</sup> • Maureen Higgins<sup>2</sup> • Tarik A. Mohamed<sup>1</sup> • Nahla S. Abdel-Azim<sup>1</sup> • Paul W. Pare<sup>3</sup> • Albena T. Dinkova-Kostova<sup>2</sup>

<sup>1</sup>*Phytochemistry Department, National Research Centre, 33 El Bohouth st. P.O. Box 12622, Dokki, Giza, Egypt.*

<sup>2</sup>*Jacqui Wood Cancer Centre, Division of Cancer Research, School of Medicine, University of Dundee, Dundee, DD1 9SY, Scotland, UK.*

<sup>3</sup>*Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409, USA.*

---

**Abstract:** Medicinal plants from the Egyptian Sinai Peninsula are widely used in traditional Bedouin medicine to treat a range of conditions including cancer, and as such are a promising resource for novel anti-cancer compounds. To achieve the scientific justification of traditional uses and/or to recommend the use of those plants as medicinal herbs for cancer chemoprevention, a group of eleven Sinai plants of different species that belong to three families (Asteraceae, Lamiaceae, and Euphorbiaceae) were biologically screened for cancer preventive activity using the chemoprevention marker enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1). Among the fractions assayed, a solvent extract from *Pulicaria incisa* had potent NQO1 inducing activity. Further analysis of the mechanism of induction revealed the concentration dependent stabilization of the transcription factor NRF2 and a coordinate upregulation of the NRF2-dependent enzymes NQO1, hemeoxygenase 1 (HO-1) and glutathione *S*-transferase pi (GSTPi). These results establish *P. incisa* as a promising target for future phytochemical characterization for cancer preventive components.

---

**Keywords** Chemoprevention • NRF2 • NQO1 • HO-1 • GST • *Pulicaria incisa* • Asteraceae

\*Corresponding author.

Tel.: +20-1090221769; Fax: 20-233370931; E-mail: n1ragab2004@yahoo.com.

## Introduction

Medicinal plants have been used for thousands of years as traditional treatments, and natural products (NPs) from these plants are still the basis for most modern medicines. Additionally, current studies of these traditional medicines continue to show that they are a rich source of biologically active compounds for the development of new pharmaceutical drug leads [1-3]. The awareness that one (or more) chemical entity within the plant material is responsible for a particular pharmacological action and can be isolated for the use as single agent began in the 19th century in the context of the emerging natural science-based medicine and pharmacy [4]. With the vast number of plant species present on the planet, since only *ca.* 1% have been characterized with respect to their phytochemical composition, there is a great potential for novel drug discovery. Interestingly in many under developed countries, traditional medicines directly extracted from plants is recognized as a rich source of drugs for primary healthcare needs [5].

The Sinai Peninsula is an epicentre of medicinal plants in the Arabian Desert. Such plant species are referred to as medicinal plants if they have been observed to have biological activity for humans and/or animals or are aromatic plants. The distribution, utilization in folk medicine and active constituents of medicinal plants in Sinai continue to attract the attention of many ecologists, taxonomists and phytochemists [6-11]. The arid environmental conditions combined with human activity have had a significant impact on the abundance, diversity and distribution of endemic medicinal plants [11-14]. South Sinai contains 472 plant species including 19 Egyptian endemic species, 115 of medicinal interest, and about 170 species used in folk medicine [15]. Wild plant species in Sinai, Egypt were selected for this study based on the unique ecosystem giving rise to great plant diversity. Geographically, the region is characterized by large variations in landforms, water resources, aridity conditions and temperatures (i.e. cold winters, hot summers, low precipitation and high evaporation). As medicinal plants have been documented as a source of novel metabolites of pharmaceutical and agricultural importance, this study also aimed to give a deeper insight into the ‘enigma’ of endemic wild plants that are usually used by Bedouin of Sinai Peninsula. The findings will be instructive in identifying novel bioactive compounds from selected plants.

While chemotherapeutic agents are initially cytotoxic to a variety of tumour cell lines and are effective in treating cancer, drug resistance often develops and is a major cause for discontinuation of a particular therapy [16]. Based on the classified stages of the

carcinogenesis process, the field of cancer chemoprevention is defined as the use of relatively non-toxic chemical agent (natural or synthetic) to inhibit, arrest or reverse the carcinogenesis at early stages [17-19]. The updated WHO traditional medicine strategy for the period 2014–2023 devotes more attention than its predecessor to prioritizing health services and systems, including traditional and complementary medicine products, practices and practitioners. Several studies on the traditional use of Egyptian plants have documented various biological activities, including antibacterial and antifungal [20, 21], antiviral [22], cytotoxic [21, 23], and antioxidant [24].

The activation of the transcription factor NF-E2 p45-related factor 2 (NRF2) and the resulting induction of its related gene products have been shown to play a key role in cancer chemoprevention. Many cytoprotective proteins such as NAD(P)H:quinone oxidoreductase 1 (NQO1), hemoxygenase-1 (HO-1) and glutathione S transferases (GSTs) that catalyze the detoxification of procarcinogens and reduce of the endogenous levels of toxic reactive oxygen species are commonly transcribed via the NRF2 signalling [25]. NQO1 induction has been used as a marker for the chemopreventive potential of natural products and synthetic chemicals [26].

As part of our continuing work to investigate and biologically evaluate the Egyptian wild and medicinal plants, the present study aimed to document the ethno-medicinal and/or recommended uses [27-33]. Here In the present study, eleven plants from different families were solvent extracted with either [MeOH:H<sub>2</sub>O (7:3) and CH<sub>2</sub>Cl<sub>2</sub>: MeOH (1:1)]. A total of twenty two extracts were tested using the NQO1 activity screening assay.

## **Materials and Methods**

### **Plant material and extraction**

Eleven plants species were collected in June 2014, from South Sinai, Egypt and aerial parts were air-dried. Plants were identified and voucher specimens have been deposited in the Herbarium of Saint Catherine protectorate, Egypt. The collection was performed under the permission of Saint Catherine protectorate for scientific purposes and officially permission was granted from the National Research Center.

Two batches of aerial parts (100 g) of each plant were powdered and extracted with CH<sub>2</sub>Cl<sub>2</sub>-MeOH (1:1) and MeOH-H<sub>2</sub>O (7:3) at room temperature. The filtrate solvents extract were

concentrated *in vacuo* using rotator evaporator to obtain a crude extract, resulting in twenty-two crude extracts containing compounds with varying polarity.

High performance liquid chromatography (HPLC) of the non polar extract from *Pulicaria incisa* (PI 1:1), which exhibited the most potent NQO1 inducing activity, was performed on an Agilent pump equipped with an Agilent-1200 HPLC instrument using variable wavelength UV detector at 220 and 254 nm using analytical column YMC-Pack ODS-A (250 x 4.6 mm i.d.) (Fig. S1).

### **Cell culture**

Murine hepatoma Hepalclc7 cells (obtained from ATCC®, USA) were grown in  $\alpha$ -MEM supplemented with 10% (v/v) fetal bovine serum (heat-and charcoal-inactivated). Cells were routinely maintained in a humidified incubator at 37 °C, 5% CO<sub>2</sub>.

### **Evaluation of NQO1 inducer activity**

A quantitative NQO1 microtiter plate assay was employed based on previously published protocols [26, 34]. For each experiment, cells (10,000 per well) were plated in 96-well plates. After 24 h, the cell culture medium was replaced with fresh medium containing plant extracts and the cells were incubated for an additional 48 h. Eight replicate wells of seven (0-50  $\mu$ g/ml) serial dilutions of each plant extract were tested. Plant extracts were prepared as stock solutions in DMSO, and then freshly diluted in the cell culture medium as 1:1000. The final concentration of DMSO in the medium was maintained at 0.1% (v/v). At the end of the 48 h exposure time, cells were lysed for 30 min at 25 °C in digitonin (0.8 g/L, pH 7.8). The specific activity of NQO1 was evaluated in cell lysates using menadione as a substrate. Protein concentrations were determined in each well by the BCA protein assay (Thermo Scientific, UK). Sulforaphane, a potent classical NQO1 inducer [35] was used as a positive control.

### **Western Blotting for NRF2 and related targets**

For protein expression analysis of the potent NQO1 inducer extract, Hepalclc7 cells (overnight incubated monolayers of  $3 \times 10^5$  cells/well in 6-well plates) were treated with three concentrations of the plant extracts (3.125, 12.5 and 50  $\mu$ g/ml) or vehicle (0.1%

DMSO). Sulforaphane (positive control) in 0.1% acetonitrile (ACN) was used as a known inducer of NRF2 and related target proteins. Cell lysates were prepared in RIPA buffer containing PMSF and protease inhibitors cocktail. Total proteins were measured using BCA assay. Samples were loaded into 10% Bis-Tris gel and run in 1x MOPS buffer at 150 Volts for 45 minutes. Resolved proteins were then transferred to nitrocellulose membranes at 60 Volts for 2 h. Membranes were blocked in 5% non-fat milk in PBST for 1 h at 25 °C and then probed overnight (4°C) with primary antibodies against NRF2, NQO1, HO-1 and GST Pi (all are generous gifts from John Hayes, University of Dundee). Membranes were probed for  $\beta$ -actin as loading control. After three washes in PBST (10 min each), membranes were probed with appropriate secondary antibodies for 1 h at 25 °C, washed three times in PBST and then developed using Enzyme Chemiluminescence. Protein bands were visualised on BioRad Chemidoc<sup>®</sup> Imager.

## Results & Discussion

Selected plants from Sinai were collected according to endemic and/or ethnobotanical uses in traditional medicines. The plants assayed included: *Achillea fragrantissima* (**1, AF**), *Artemisia judica* (**2, AJ**), *Ballota undulata* (**3, BU**), *Euphorbia santa-catherine* (**4, ES**), *Phlomis aurea* (**5, PA**), *Pulicaria incisa* (**6, PI**), *Stachys aegyptiaca* (**7, SA**), *Pulicaria undulata* (**8, PU**), *Seriphidium herba-alba* (**9, SH**), *Teucrium polium* (**10, TP**), *Tanacetum sinaicum* (**11, TS**). Photographs of these plants growing in their wild habitat are displayed in Fig.1.

Hepa1c1c7 cells were incubated for 48 h with increasing concentrations (0-50 µg/ml) of each extract, and the activity of the NQO1 marker enzyme was quantified using the Prochaska assay. *P. incisa* (PI 1:1) resulted in the highest concentration-dependent induction of NQO1 specific enzyme activity, as shown in Fig.2 and Table 1, where its CD value (the concentration needed to double the NQO1 activity by 2-fold relative to the solvent control) was 3.2 µg/ml. At 50 µg/ml, the highest concentration tested, the PI 1:1 extract led to a robust 8-fold induction of the NQO1 specific enzyme activity.

–Fig. 1–

In contrast to the potent NQO1 inducer activity produced by the non-polar *P. incisa* extract (PI 1:1), the methanol:water (7:3) extract of this *Pulicaria* species (PI 70) caused a much weaker inducer activity (1.9 fold increase of the NQO1 activity over vehicle control) at the highest concentration of 50 µg/ml. Dose-dependent increases of NQO1 activity was found with extracts from *P. undulata* (PU 1:1), *S. herba-alba* (both SH 1:1 and SH 70), and *T. sinaicum* (both TS 1:1 and TS70 extracts) with CD values ranged between 5.0 and 10.0 µg/ml (Fig.2 and Table 1). Moderate NQO1 inducer activities (CD value between 12.0 and 16.0 µg/ml) was recorded for extracts from *A. fragrantissima* (AF 1:1 and AF 70) and *A. judica* (AJ 1:1). Some of the tested extracts were weak NQO1 inducers, including *B. undulata* (BU 1:1, CD=22 µg/ml), *E. santa-catherine* (ES 70, CD=31 µg/ml), *T. polium* (TP 1:1, CD=28) and *S. aegyptiaca* (SA 70, CD=50 µg/ml). The rest of tested plant extracts were devoid of pronounced NQO1 inducer activity so that they failed to reach a CD value even at 50 µg/ml, the highest concentration tested.

Because the NQO1 screening assay showed that the PI 1:1 extract has the most potent concentration-dependent induction of NQO1 specific enzyme activity (Fig.2 and Table 1), we focused our subsequent studies on this extract. The gene expression of NQO1 is mediated primarily through the activity of transcription factor NRF2. Under basal conditions, NRF2 is bound to its main negative regulator, Kelch-like ECH-associated protein 1 (KEAP1), which serves as a substrate adaptor protein for a Cullin-3/Rbx1 ubiquitin ligase and mediates the continuous ubiquitination and proteasomal degradation of NRF2. KEAP1 is also a cysteine-based sensor for various endogenous and exogenous sulfhydryl-reactive small molecules (termed inducers), such as the isothiocyanate sulforaphane. Such compounds react and chemically modify the cysteine sensor(s) of Keap1 and disable its substrate adaptor activity, resulting in a rapid stabilization of NRF2. Therefore we next examined the protein levels of NRF2 after 4 h of exposure of Hepa1c1c7 cells to the PI 1:1 extract. As expected based on the NQO1 inducer activity, treatment with the PI 1:1 extract led to a concentration-dependent increase in the protein levels of NRF2 (Fig.3A).

–Fig. 2, Table 1–

Simultaneous induction of the expression of HO-1, another NRF2-target protein, was also evident at the 4 h time point. In agreement with the enzyme activity assay, the levels of NQO1 protein were also dose-dependently upregulated by exposure to the PI 1:1 extract for 24 h (Fig. 3B). Finally, we examined levels of GST-Pi, another member of the NRF2-regulated cytoprotective proteome after 24 h of treatment with PI 1:1. GST-Pi was found to be upregulated coordinately with HO-1 and NQO1. Notably, the increases in HO-1 and NQO1 were much more pronounced than the increase in GST-Pi (Fig.3C), further supporting the use of NQO1 as a sensitive marker for NRF2 activation.

–Fig. 3–

Our study has uncovered the value of the *P. incisa* non-polar extract (PI 1:1) as an activator of the NRF2-dependent enzyme NQO1. These interesting results warrant further fractionation and isolation of the chemical constituents of this plant extract using bioassay-guided isolation schemes, which is underway.



## **Acknowledgments**

This project was supported financially by Cancer Research UK (C20953/A18644), the Welch Foundation (D-1078), and National research Centre Egypt. We thank John D. Hayes (University of Dundee) for antibodies.

## References

- [1] Phillipson JD (2001) Phytochemistry and medicinal plants. *Phytochem* 56: 237-243.
- [2] Balunas MJ, Kinghorn AD (2005) Drug discovery from medicinal plants. *Life Sci.* 78: 431–441.
- [3] Saxena M, Saxena J, Nema R, Singh D, Gupta A (2013) Phytochemistry of medicinal plants. *J Phamacogn Phytochem* 1: 168–182.
- [4] Sprouse AA, Van Breemen RB(2016). Pharmacokinetic interactions between drugs and botanical dietary supplements. *Drug Metab Dispos* 44: 162-171.
- [5] Kim H-S (2005) Do not put too much value on conventional medicines. *J Ethnopharmacol* 100: 37–39.
- [6] Bailey C, Danin A (1981) Bedouin plant utilization in Sinai and the Negev. *Econ Bot* 21: 45-162.
- [7] Boulos L (1983) Medicinal plants of North Africa. References publication, Inc. Algonac, Michigan.pp. 286.
- [8] Bown D (1995) Encyclopedia of Herbs and their uses. Dorling Kindersley, London.
- [9] Batanouny KH, Aboutabl E, Shabana M, Soliman F (1999) Wild Medicinal plants in Egypt. An inventory to support conservation and sustainable use. The Palm press, Cairo, Egypt.pp. 207.
- [10] Hanafi Y, Abdel-Wahab M (2000) Wild medicinal plants in Sinai. *Arabian Gulf of Est. Egypt. (In Arabic)* pp. 337.
- [11] Abd El-Wahab RH, Zaghloul MS, Moustafa AA (2004) Conservation of medicinal plants in St. Catherine Protectorate, South Sinai. I. Evaluation of ecological status and human impact. Proceedings of first International conference on Strategy of Egyptian Herbaria, Giza, Egypt. 231-251.
- [12] Zaghloul MS (1997) Ecological studies on some endemic plant species in South Sinai, Egypt. M.Sc. Thesis, Faculty of Science, Suez Canal University.

- [13] Moustafa AA, Zaghoul MS, Abd El-Wahab RH, Shaker M (2001) Evaluation of plant diversity and endemism in Saint Catherine Protectorate, South Sinai, Egypt. *Egypt J Bot* 4: 123-141.
- [14] Batanouny KH (1983) Human impact on Desert Vegetation. In: *Man's Impact on Vegetation*, Holzner, W., Werger, M.J.A. and Ikusima, I. (eds.), Dr. W. Junk Publishers, London. pp. 380.
- [15] Fayed A, and Shaltout K (2004) Conservation and sustainable use of Medicinal plants in arid and semi-arid eco-systems project, Egypt (GEF, UNDP) (project no: 12347/12348), Flora of Saint Catherine protectorate, and Floristic Survey of the Mountainous Southern Sinai: Saint Katherine Protectorate, 2004.
- [16] Hurley LH (2002) DNA and its associated processes as targets for cancer therapy. *Nat Rev Cancer*, 2: 188–200.
- [17] Surh YJ (2003) Cancer chemoprevention with dietary phytochemicals. *Nat Rev Cancer* 3:768-780.
- [18] Manson MM, Gescher A, Hudson EA, Plummer SM, Squires MS, Prigent SA (2000) Blocking and suppressing mechanisms of chemoprevention by dietary constituents. *Toxicol Lett* 112-113: 499-505.
- [19] Zhao CR, Gao ZH, Qu XJ (2010) Nrf2-ARE signaling pathway and natural products for cancer chemoprevention. *Cancer Epidemiol* 34:523-533.
- [20] Khafagi IK (1999) Screening in vitro cultures of some sinai medicinal plants for their antibiotic activity. *Egypt J of Microbiol* 34: 613-627.
- [21] Kuete V, Wiench B, Hegazy ME, Mohamed TA, Fankam AG, Shahat AA, Efferth T (2012) Antibacterial activity and the cytotoxicity of the organic extracts from 16 selected medicinal plants of Egypt. Antibacterial activity and cytotoxicity of selected Egyptian medicinal plants. *Planta Med* 78: 193-199.
- [22] Soltan MM, Zaki AK (2009) Antiviral screening of forty-two Egyptian medicinal plants. *J Ethnopharmacol* 126:102-107.

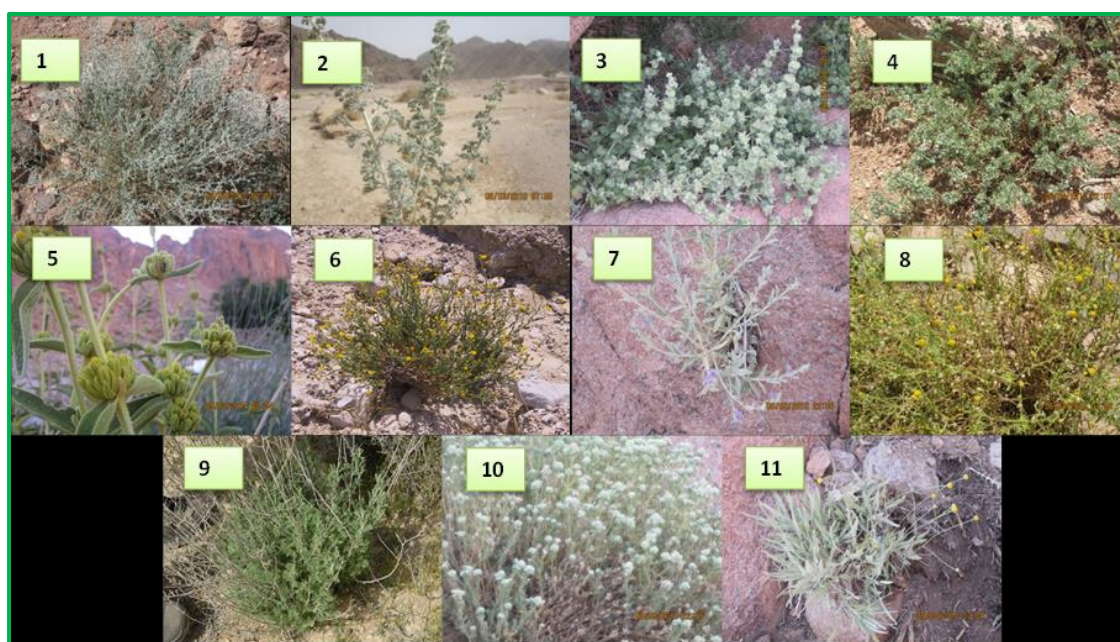
- [23] El-Seedi HR, Burman R, Mansour A, Turki Z, Boulos L, Gullbo J, Göransson U (2013) The traditional medical uses and cytotoxic activities of sixty-one Egyptian plants: Discovery of an active cardiac glycoside from *Urginea maritima*. J of Ethnopharmacol 14: 5746–757.
- [24] Eissa TF, González-Burgos E, Carretero ME, Gómez-Serranillos MP (2014) Compositional analysis and in vitro protective activity against oxidative stress of essential oils from Egyptian plants used in traditional medicine. Nat Prod Commun 9: 1377-1382.
- [25] Dinkova-Kostova AT, Kostov RV (2012) Glucosinolates and isothiocyanates in health and disease. Trends Mol Med 18:337-47.
- [26] Fahey JW, Dinkova-Kostova AT, Stephenson KK, Talalay P (2004) The “ Prochaska ” microtiter plate bioassay for inducers of NQO1. Methods Enzymol 382: 243–258.
- [27] Elmasri W, Hegazy MEF, Mechref Y, Paré PW (2016). Structure-antioxidant and anti-tumor activity of *Teucrium polium* phytochemicals. Phytochem Lett 15: 81–87.
- [28] Hegazy MEF, Nakamura S, Tawfik WA, Abdel-Azim NS, Abdel-Lateff A, Matsuda H, Paré PW (2015). Rare hydroperoxyl guaianolide sesquiterpenes from *Pulicaria undulata*. Phytochem Lett 12, 177–181.
- [29] Hegazy MEF, Ibrahim AY, Mohamed TA, Shahat AA, El-Halawany AM, Abdel-Azim, NS, Alsaid M.S., Paré PW (2016) Sesquiterpene Lactones from *Cynara cornigera*: Acetyl Cholinesterase Inhibition and In Silico Ligand Docking. Planta Med 82: 138–146.
- [30] Hegazy MEF, Hamed AR, Mohamed TA, Debbab A, Nakamura S, Matsuda H, Paré PW (2015) Anti-inflammatory sesquiterpenes from the medicinal herb *Tanacetum sinaicum*. RSC Adv 5, 44895–44901.
- [31] Hegazy MF, Matsuda H, Nakamura S, Yabe M, Matsumoto T, Yoshikawa M (2012) Sesquiterpenes from an Egyptian Herbal Medicine, *Pulicaria undulate*, with Inhibitory Effects on Nitric Oxide Production in RAW264.7 Macrophage Cells. Chem Pharm Bull 60: 363–370.

- [32] Abdel-Azim NS, Shams KA, Shahat AA, ElMissiry MM, Ismail SI, Hammouda FM (2011). Egyptian Herbal Drug Industry: Challenges and Future Prospects. *Res J Med Plant* 5: 136-144.
- [33] Ali SK, Hamed AR, Soltan MM, El-Halawany AM, Hegazy UM, Hussein AA (2016) Kinetics and molecular docking of vasicine from *Adhatoda vasica*: An acetylcholinesterase inhibitor for Alzheimer's disease. *S Afr J Bot* 104:118-124.
- [34] Prochaska HJ, Santamaria AB (1988) Direct measurement of NAD(P)H:quinone reductase from cells cultured in microtiter wells: a screening assay for anticarcinogenic enzyme inducers *Anal Biochem* 169: 328-336.
- [35] Zhang Y, Talalay P, Cho CG, Posner GH (1992) A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proc Natl Acad Sci* 89: 2399 -2403.

**Table 1.NQO1 Inducer Potency\* of 22 Plant extracts for 11 plants.**

<b>Extract</b>	<b>CD (<math>\mu\text{g/ml}</math>)</b>
AF 1:1	12.6
AF 70	13.0
AJ 1:1	16.0
AJ 70	N.R.
BU 1:1	22.0
BU 70	N.R.
ES 1:1	N.R.
ES 70	31.0
PA 1:1	N.R.
PA 70	N.R.
PI 1:1	3.2
PI 70	N.R.
PU 1:1	5.9
PU 70	N.R.
SA 1:1	50.0
SA 70	N.R.
SH 1:1	6.0
SH 70	5.9
TP 1:1	28.0
TP70	N.R.
TS 1:1	10.0
TS 70	9.0

\*Potency is expressed as the concentration of test sample needed to double the NQO1 specific enzyme activity. N.R.=not reached up to extract concentration of 50  $\mu\text{g/ml}$ .



**Fig.1 Images of assayed Egyptian plants in their original habitat:** *Achillea fragrantissima* (1, AF), *Artemisia judica* (2, AJ), *Ballota undulata* (3, BU), *Euphorbia santa-catherine* (4, ES), *Phlomis aurea* (5, PA), *Pulicaria incisa* (6, PI), *Stachys aegyptiaca* (7, SA), *Pulicaria undulata* (8, PU), *Seriphidium herba-alba* (9, SH), *Teucrium polium* (10, TP) and *Tanacetum sinaicum* (11, TS).

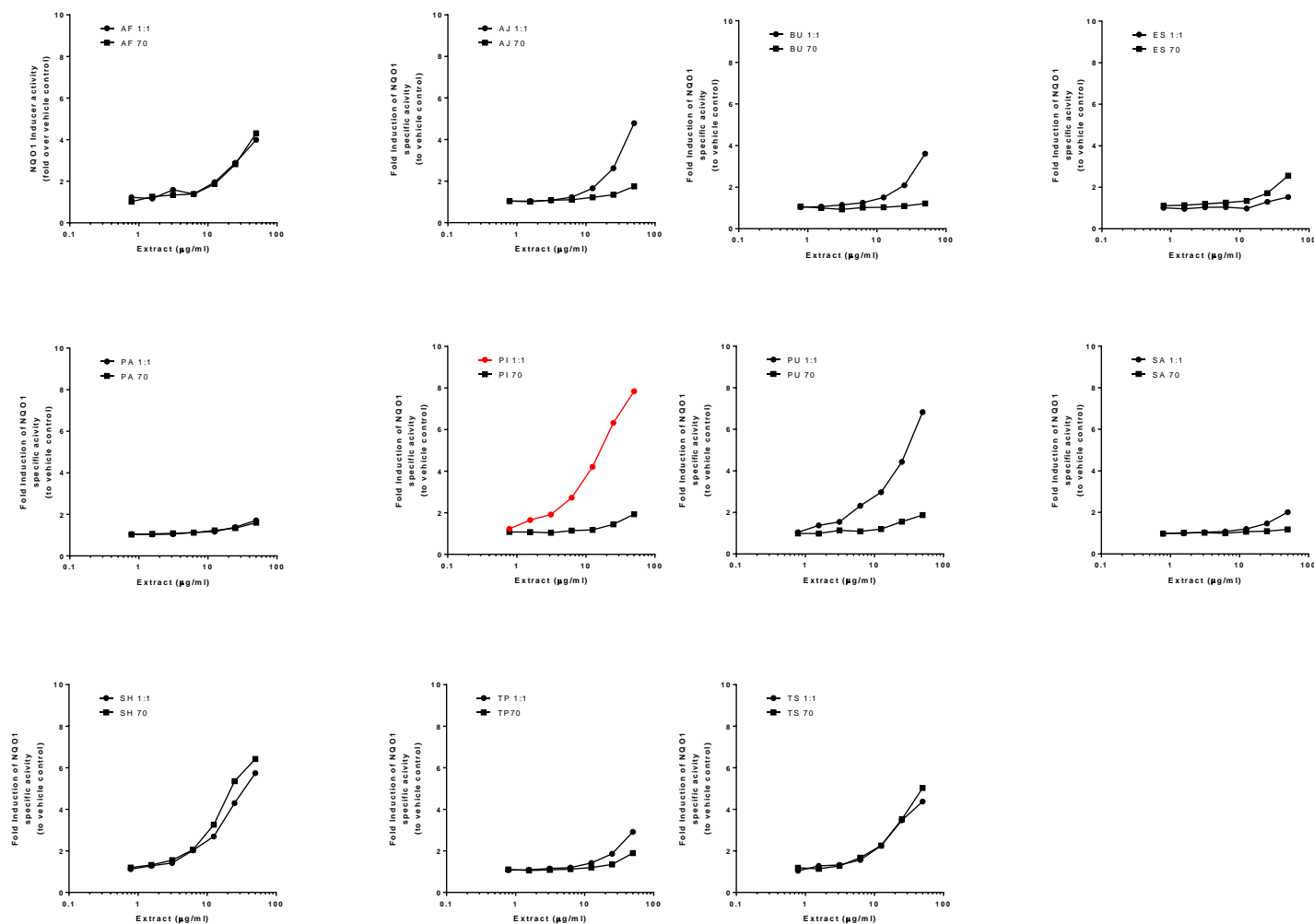


Fig. 2. Dose response of NQO1 inducer activity of 22 extracts from 11 plant species.



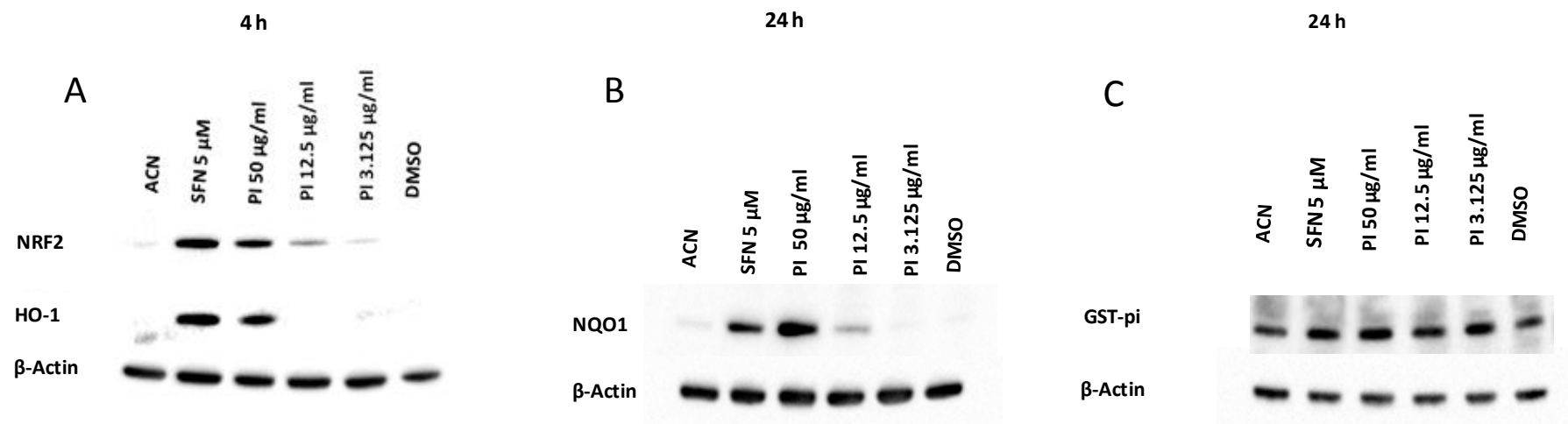


Fig.3. Stabilization of NRF2 and upregulation of its related cytoprotective proteins by the PI 1:1 plant extract in Hepa1c1c7 cells.

## Supporting information:

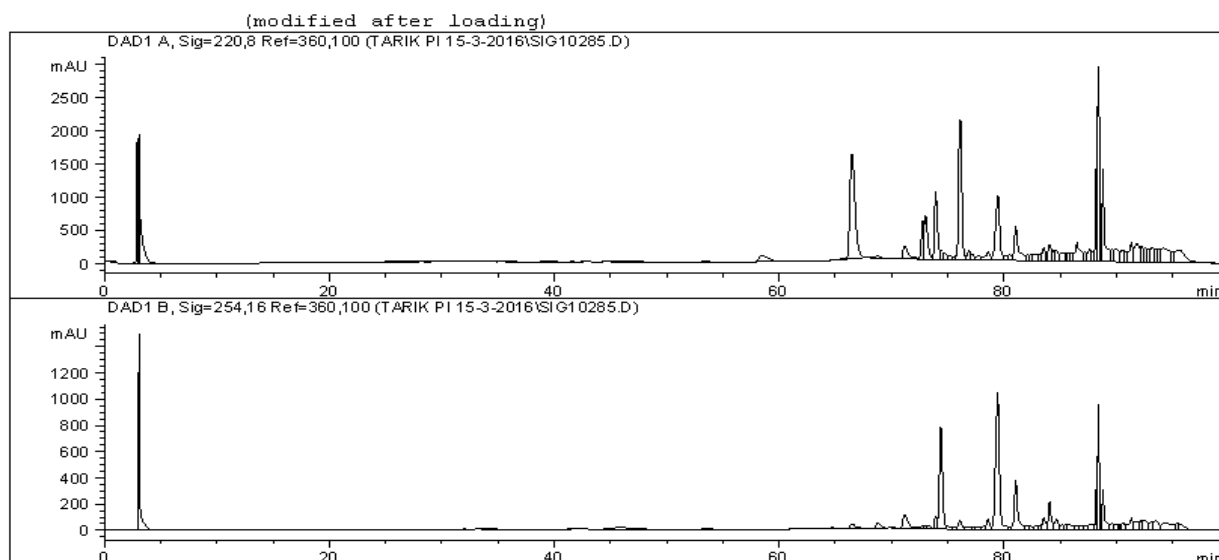


Fig. S1. Typical chromatograms of active plant extract, *Pulicaria incisa* ( $\text{CH}_2\text{Cl}_2$ : MeOH (1:1)). The separation conditions as follow: chromatogram column, ODS column (4.6×200 mm, 5 $\mu\text{m}$ ); column temperature, 30°C; injection volume, 10 $\mu\text{L}$ ; elution was performed at a flow rate of 1 ml/min, using as mobile phase a mixture of water (A) and methanol (B). The samples were eluted by the following gradient: 95% A and 5% B as initial conditions, 70% A and 30% B for 25 min, 65% A and 35% B for 25 min, 30% A and 70% B for 15 min, 0% A and 100% B for 5 min and, finally, 95% A and 5% B for 5 min. Detection was performed at 220 and 254 nm.